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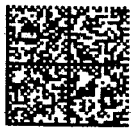

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/241,636	02/02/1999	ELLEN M. HEATH	5253	8977

7590 04/26/2005

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EXAMINER	
GOLDBERG, JEANINE ANNE	
ART UNIT	**PAPER NUMBER**
1634	

DATE MAILED: 04/26/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/241,636	HEATH ET AL.	
	Examiner	Art Unit	
	Jeanine A. Goldberg	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03 February 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *63-109* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *63-109* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1)☒ Notice of References Cited (PTO-892)

2)☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3)☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.

4)☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.

5)☐ Notice of Informal Patent Application (PTO-152)

6)☐ Other: _____.

DETAILED ACTION

1. This action is in response to the papers filed February 3, 2005. Currently, claims 63-109 are pending.

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on February 3, 2005 has been entered.

3. This action contains new grounds or rejection necessitated by amendment.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 99-10 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A) The phrase "at least about" because the metes and bounds of the invention are not clear. As the CAFC noted, and affirmed, regarding the district court determination of this phrase in Amgen Inc. v. Chugai Pharmaceutical Co. Ltd. (CA FC) 18 USPQ2d 1016 at page 1031 "the court held the "at least about" claims to be invalid for indefiniteness." Here too, the situation is that there is close prior art, applied as a

102(b) for a lower limit value, and the claim is indefinite with regard to the values encompassed.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

5. Claims 63-67, 69-73, 81-82, 84, 87-90, 101-109 are rejected under 35 U.S.C. 103(a) as being unpatentable over Harvey et al. (US Pat. 5.939,259, August 1999) in view of Rudi et al. (WO 98/51693, November 19, 1998).

Harvey teaches a method and device for collecting and storing clinical samples for genetic analysis. Harvey teaches a process for characterizing DNA by isolating nucleic acids which comprises contacting a biological material with a solid support treated with a lysing reagent (i.e. a absorbent material that is impregnated with chaotropic salt); b) treating the biological material with a DNA purifying agent (i.e. water and vortex), c) purifying the DNA from the remainder (i.e. supernatant) d) analyzing the purified DNA (i.e. PCR reactions and electrophoresis)(col. 5, lines 25-55). Moreover, Harvey specifically teaches fabricating an absorbent material with a roll of 903 paper which is impregnated with guanidine thiocyanate solution having a concentration between 0.5M and 5.0 M. The paper is allowed to dry (col. 5, lines 10-22). Harvey teaches isolating DNA from fecal sources, saliva sources, and whole blood sources

(limitations of Claim 71-72, 89). Specifically, two separate squares of 903 paper are exposed to samples, one paper treated and the other paper untreated (limitations of Claim 90). The samples was allowed to dry and the papers was transferred to a centrifuge tube containing water and vortexed (col. 5, lines 30-35)(limitations of Claim 69). The paper was further transferred to a second centrifuge tube containing water and placed on a heating block at 95 degrees for 30 minutes (col. 5, lines 35-40)(limitations of Claim 70). The supernatant from each sample was amplified and analyzed by electrophoresis on a polyacylamide gel which were visualized by silver staining (Example 6). Moreover Harvey specifically claims a method for collecting nucleic acids from a whole blood source by contacting a whole blood source with an adsorbent material that has a chaotropic salt impregnated, allowing the source to be absorbed on the absorbent material and eluting the nucleic acids into a solution that can be used in a nucleic acid amplification process (col. 8)(limitations of Claim 88, 101-102).

Harvey does not specifically teach a method which uses an RNA digesting enzyme.

However, Rudi et al. (herein referred to as Rudi) teaches a method of solid-phase nucleic acid isolation. Rudi teaches "if it is desired to remove RNA from DNA, this may be achieved by destroying the RNA." Rudi teaches that this may be done by the addition of an RNAase or an alkali such as NaOH (page 15, lines 34-36)(limitations of Claim 67). Rudi further teaches that any cell including prokaryotic and eukaryotic cells, mammalian and non-mammalian cells, bacteria may be used (page 4). Further samples comprising these materials may include foods, clinical and environmental

samples including soil, water or food samples (limitations of Claims 73). Moreover, Rudi teaches analyzing the nucleic acids following isolation (page 17). Rudi discusses detecting nucleic acids using hybridization, PCR, minisequencing (limitations of Clams 82, 84, 87, 88)

Therefore, it would have been prima facie obvious, at the time the invention was made to have modified the solid phase lysis and detection method of Harvey to include contacting the solid phase with an RNAse. Harvey specifically teaches that if it is desired to remove RNA from DNA this may be done by addition of an RNAase (page 15). Therefore, the ordinary artisan would have been motivated to have removed RNA from a sample to enable detection of DNA. The ordinary artisan would have been motivated to have added a RNAase to the lysing reagent to enable the rapid detection of DNA without an additional step. The ordinary artisan would have had a reasonable expectation of success for modifying the impregnated solid support comprising a lysing reagent with an RNAase to enable a simultaneous method for lysis and removal of RNA.

Further, the ordinary artisan would have been motivated to have used any of the samples taught in the art for the efficient detection of nucleic acids. Rudi teaches many sources and samples may be used. The ordinary artisan would have been motivated to have used any of the variety of samples taught as successful by Rudi for the express benefit of analyzing a variety of samples.

Additionally, Rudi teaches the following isolation the purified nucleic acid may be analyzed using sequencing, minisequencing, PCR, hybridization for example. The

ordinary artisan would have been motivated to have taken the purified nucleic acid obtained by Harvey in view of Rudi and further analyzed the sample to obtain information regarding the nucleic acid. While Harvey teaches the nucleic acid may be used for PCR reactions, the additional analysis methods taught by Rudi would have a reasonable expectation of success to analyze the nucleic acids purified. Thus, the ordinary artisan would have been motivated to have analyzed the nucleic acids using any of the know methods depending on the desired results of the analysis.

6. Claims 63-67, 69, 71-73, 81-90, 101-109 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boom et al (5,234,809) in view of Shieh (US Pat. 6,054,039, April 2000) in view of Rudi et al. (WO 98/51693, November 19, 1998).

Boom teaches methods of characterizing DNA. Boom discloses a process which involved contacting the biological material that contains DNA with a solid support that had been treated with a lysing reagent (i.e. a chaotropic substance), treating the biological material with a purifying reagent, and purifying the DNA (col. 4, lines 3-59). The chaotropic substance (lysing reagent) is contacted with silica particles (solid support). The biological material is then treated with purifying reagents, and the remainder of the biological matter is purified (washing buffer, alcohol washing solution and acetone. Additionally, the process described by Boom produces DNA which can be further used to "demonstrate NA sequences by means of an amplification method such as the PCR method...." (col.4, lines 48-50)(limitations of claims 87-88). Boom teaches that the Gus SCN (i.e. the lysis reagent) is added to the solid support (i.e. the silica

beads) prior to addition of biological material. Boom teaches silicon dioxide supports, nitrocellulose supports, latex particles (col. 5-6)(limitations of claims 90). As described in Boom, the DNA may be eluted from the solid support by means of an eluting reagent (col.4, line 33). Boom teaches an eluting reagent can be TE buffer, aqua bidest or PCR buffer. Boom further teaches the process where in the solid support is contained in a single vessel (col.4, lines 34-36) (limitations of claims 69). Boom demonstrates the use of isolating nucleic acids from a nucleic acid-containing biological material (col. 1, lines 10-20). The biological material stated includes tissues, cell cultures, blood, urine, and saliva (body fluids)(limitations of claims 71-72). The nucleic acid was taught to be examined by gel electrophoresis (col. 10, lines 13-24) (limitations of claims 82-85). This method may be used for characterizing the biological material and monitoring impurities. Yields were also taught in example A1 (col. 12, lines 46-48)(limitations of claim 83). Eluted DNA was treated with a restriction enzyme, electrophoresed and visualized (col. 12 65-68) (limitations of claims 86). Boom also teaches hybridization analysis of the isolated nucleic acids (col. 9, lines 19-21)(limitations of claims 87-88). Boom teaches a method which can "provide a process with which nucleic acid can be isolated immediately..." (col. 1, lines 64-67). Boom teaches lysis buffers containing Tris (buffer), aqua bidest, GuSCN, and EDTA (col. 6, lines 39-68).

Boom does not explicitly teach using a solid support in which the lysing reagent is bound, and unbound lysing reagent is removed prior to the contacting of the biological treatment.

However, Shieh teaches a method for lysing cells. Specifically, Shieh teaches the modification of a membrane strip to produce lysis of red blood cells that contacts it (col. 6, lines 17-20). Shieh teaches that membranes such as polymer treated glass fibers, polyamides, cellulose, polyesters may be used (col. 10, lines 42-57)(limitations of Claims 90). Shieh teaches preparing a lysing component by treating the membranes with a lysing agent (col. 10, lines 65-67). Shieh teaches that lysing agents included Mega 8, Triton X, lauryl sulfate salts, TEA sals, sodium salt, among numerous others (col. 11, lines 1-10)(limitations of Claims 94). Furthermore, Shieh teaches that the lysing agent may be coated onto the membrane by any method used in the art for coating solutions onto films such as dip coating an aqueous solution or disperson of the lysing solution onto the membrane and allowing to dry (col. 11, lines 10-20). As provided in Example 1D, a cell lysing membrane was prepared (col. 12, lines 8-18). Furthermore, Shieh teaches that "this component caused lysis of whole blood when it passed across the membrane (col. 14, lines 31-33). Shieh teaches that the method and sensor may be used "on the spot: at home, in a physicians office or in a hospital room". Shieh also teaches the sensor is low cost and disposable (col. 15, lines 55-60).

Further, Rudi et al. (herein referred to as Rudi) teaches a method of solid-phase nucleic acid isolation. Rudi teaches "if it is desired to remove RNA from DNA, this may be achieved by destroying the RNA." Rudi teaches that this may be done by the addition of an RNAase or an alkali such as NaOH (page 15, lines 34-36)(limitations of Claim 67). Rudi further teaches that any cell including prokaryotic and eukaryotic cells, mammalian and non-mammalian cells, bacteria may be used (page 4). Further

samples comprising these materials may include foods, clinical and environmental samples including soil, water or food samples (limitations of Claims 73). Moreover, Rudi teaches analyzing the nucleic acids following isolation (page 17). Rudi discusses detecting nucleic acids using hybridization, PCR, minisequencing (limitations of Clams 82, 84, 87, 88)

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Boom, which characterizes DNA using a solid support, lysing reagent and a biological material with a pre-treated membrane of Shieh. Shieh teaches that biological samples may be lysed using a pre-treated membrane such that lysis is caused when a sample is passed across the membrane. The ordinary artisan would have been motivated to have produced a solid support which was pre-treated with a lysing reagent, as taught by Shieh, for the expected benefit taught by Shieh as low cost and disposability. The ordinary artisan would also have been motivated to have prepared the pre-treated lysing membranes, of Shieh, for use in the method, of Boom, for the expected benefit of convenience. Moreover, the skilled artisan would have had a reasonable expectation of success for analyzing DNA from a solid support that was pretreated with a lysing reagent since Boom teaches a method in which all three components, a lysing reagent, solid support and nucleic acid sample, were contacted with successful results.

Further, the ordinary artisan would have been motivated to have used any of the samples taught in the art for the efficient detection of nucleic acids. Rudi teaches many sources and samples may be used. The ordinary artisan would have been motivated to

have used any of the variety of samples taught as successful by Rudi for the express benefit of analyzing a variety of samples.

Additionally, Rudi teaches the following isolation the purified nucleic acid may be analyzed using sequencing, minisequencing, PCR, hybridization for example. The ordinary artisan would have been motivated to have taken the purified nucleic acid obtained by Harvey in view of Rudi and further analyzed the sample to obtain information regarding the nucleic acid. While Boom in view of Shieh teaches the nucleic acid may be used for PCR reactions, the additional analysis methods taught by Rudi would have a reasonable expectation of success to analyze the nucleic acids purified. Thus, the ordinary artisan would have been motivated to have analyzed the nucleic acids using any of the know methods depending on the desired results of the analysis.

Thus, the skilled artisan would have combined the teachings of Boom with the teachings of Shieh in further view of Rudi.

7. Claims 63-67, 69-85, 87-90, 94-96, 101-109 are rejected under 35 U.S.C. 103(a) as being unpatentable over Deggerdal (WO 96/18731) in view of Shieh (US Pat. 6,054,039, April 2000) in view of Rudi et al. (WO 98/51693, November 19, 1998).

Deggerdal teaches methods of characterizing DNA. Deggerdal discloses a "method of isolating nucleic acid from a sample, said method comprising contacting said sample with a detergent and a solid support, whereby soluble nucleic acid in said sample is bound to the support, and separating said support with bound nucleic acid from the sample" (pg 5, para 2). Deggerdal teaches that the "nucleic acid-containing sample may be contacted with the detergent and solid phase which may be added to

the sample prior to, simultaneously with, or subsequently to the detergent (which functions in the method to lyse)"(pg 7, para 3, lines 22-29). The solid support was contained in a vessel (pg 26, line 18)(limitations of Claim 69). Deggerdal teaches isolation of nucleic acids and the elution of the nucleic acid by heating to 65 C for 5-10 minutes (pg 12, para 3, line 3)(limitations of claims 70). The samples may be of "any material containing nucleic acid" (pg 6, para 1, line 1-3)(limitations of claims 71-73). Deggerdal teaches a method of DNA isolation from cultured cells which indicates the number of cells used as starting material (pg 33, line 1) (limitations of claims 74-76). The purified DNA was then analyzed by PCR and visualized on agarose gel electrophoresis (pg 20, lines 29-32)(limitations of Claims 80). Detection of extra bands indicated contamination (pg 17, lines 26-27)(limitations of Claims 79). The solid support was taught to be made of "glass, silica, latex or a polymeric material" (pg 9, para 3)(limitations of claim 90). Deggerdal teaches an example where cells were lysed using DNA DIRECT Dynabeads and the lysate from each sample was further characterized (pg 35, lines 6-35)(limitations of claim 77-78). Deggerdal teaches the lysing reagent as a detergent. This detergent may be supplied in simple aqueous solution (pg 8, line 7). Further any suitable buffer (Tris) is taught. The reagent may also include components such as enzymes, chelating agents and reducing agents (pg 8, lines 7-23).

Deggerdal does not explicitly teach using a solid support in which the lysing reagent is bound, and unbound lysing reagent is removed prior to the contacting of the biological treatment.

However, Shieh teaches a method for lysing cells. Specifically, Shieh teaches the modification of a membrane strip to produce lysis of red blood cells that contacts it (col. 6, lines 17-20). Shieh teaches that membranes such as polymer treated glass fibers, polyamides, cellulose, polyesters may be used (col. 10, lines 42-57)(limitations of Claims 33). Shieh teaches preparing a lysing component by treating the membranes with a lysing agent (col. 10, lines 65-67). Shieh teaches that lysing agents included Mega 8, Triton X, lauryl sulfate sals, TEA sals, sodium salt, among numerous others (col. 11, lines 1-10)(limitations of Claims 61-62). Furthermore, Shieh teaches that the lysing agent may be coated onto the membrane by any method used in the art for coating solutions onto films such as dip coating an aqueous solution or disperson of the lysing solution onto the membrane and allowing to dry (col. 11, lines 10-20). As provided in Example 1D, a cell lysing membrane was prepared (col. 12, lines 8-18). Furthermore, Shieh teaches that "this component caused lysis of whole blood when it passed across the membrane (col. 14, lines 31-33). Shieh teaches that the method and sensor may be used "on the spot: at home, in a physicians office or in a hospital room". Shieh also teaches the sensor is low cost and disposable (col. 15, lines 55-60).

Further, Rudi et al. (herein referred to as Rudi) teaches a method of solid-phase nucleic acid isolation. Rudi teaches "if it is desired to remove RNA from DNA, this may be achieved by destroying the RNA." Rudi teaches that this may be done by the addition of an RNAase or an alkali such as NaOH (page 15, lines 34-36)(limitations of Claim 67). Rudi further teaches that any cell including prokaryotic and eukaryotic cells, mammalian and non-mammalian cells, bacteria may be used (page 4). Further

samples comprising these materials may include foods, clinical and environmental samples including soil, water or food samples (limitations of Claims 73). Moreover, Rudi teaches analyzing the nucleic acids following isolation (page 17). Rudi discusses detecting nucleic acids using hybridization, PCR, minisequencing (limitations of Clams 82, 84, 87, 88)

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Deggerdal, which characterizes DNA using a solid support, lysing reagent and a biological material with the method and pre-treated membrane of Shieh. Shieh teaches that biological samples may be lysed using a pre-treated membrane such that lysis is caused when a sample is passed across the membrane. The ordinary artisan would have been motivated to have produced a solid support which was pre-treated with a lysing reagent, as taught by Shieh, for the expected benefit taught by Shieh as low cost and disposability. The ordinary artisan would also have been motivated to have prepared the pre-treated lysing membranes, of Shieh, for use in the method, of Deggerdal, for the expected benefit of convenience. Moreover, the skilled artisan would have had a reasonable expectation of success for analyzing DNA from a solid support that was pretreated with a lysing reagent since Deggerdal teaches a method in which all three components, a lysing reagent, solid support and nucleic acid sample, were contacted with successful results.

Further, the ordinary artisan would have been motivated to have used any of the samples taught in the art for the efficient detection of nucleic acids. Rudi teaches many sources and samples may be used. The ordinary artisan would have been motivated to

have used any of the variety of samples taught as successful by Rudi for the express benefit of analyzing a variety of samples.

Additionally, Rudi teaches the following isolation the purified nucleic acid may be analyzed using sequencing, minisequencing, PCR, hybridization for example. The ordinary artisan would have been motivated to have taken the purified nucleic acid obtained by Harvey in view of Rudi and further analyzed the sample to obtain information regarding the nucleic acid. While Boom in view of Shieh teaches the nucleic acid may be used for PCR reactions, the additional analysis methods taught by Rudi would have a reasonable expectation of success to analyze the nucleic acids purified. Thus, the ordinary artisan would have been motivated to have analyzed the nucleic acids using any of the know methods depending on the desired results of the analysis.

Thus, the skilled artisan would have combined the teachings of Deggerdal with the teachings of Shieh in view of Rudi.

8. Claims 95, 97-97 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boom (5,234,809) in view of Shieh (US Pat. 6,054,039, April 2000) in view of Rudi et al. (WO 98/51693, November 19, 1998) as applied to Claims 63-67, 69, 71-73, 82-90, 101-109 above, and further in view of in view of Deggerdal (WO 96/18731).

Neither Boom, nor Shieh teaches a lysing reagent which does not contain a buffer.

Deggerdal, however, teaches a lysing reagent as a detergent. This detergent may be supplied in simple aqueous solution (pg 8, line 7). Further any suitable buffer

(Tris) is taught. The reagent may also include components such as enzymes, chelating agents and reducing agents (pg 8, lines 7-23) (limitations of claims 37-41).

Therefore, it would have been prima facie obvious to one of ordinary skill at the time of the invention was made to have modified the method of Boom in view of Shieh to include the use of the lysing reagents taught in Deggerdal. The ordinary artisan would have been motivated to use the lysing reagents taught in Deggerdal because the lysing reagents taught in Deggerdal were readily available.

9. Claims 68, 99-100 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boom (5, 804,684) in view of Shieh (US Pat. 6,054,039, April 2000) in view of Rudi et al. (WO 98/51693, November 19, 1998) as applied to Claims above 63-67, 69, 71-73, 82-90, 101-109 or Deggerdal (WO 96/18731) in view of Shieh (US Pat. 6,054,039, April 2000) in view of Rudi et al. (WO 98/51693, November 19, 1998) as applied to Claims above 63-67, 69-80, 82-85, 87-90, 93-96, 101-109 or Harvey et al. (US Pat. 5.939,259, August 1999) in view of Rudi et al. (WO 98/51693, November 19, 1998) as applied to Claims 63-67, 69-73, 82, 84, 87-90, 101-109 above and further in view of Su (5,804684).

Neither Boom in view of Shieh nor Deggerdal in view of Shieh nor Harvey specifically teach the eluting reagent as specified in the claims.

However, Su teaches the elution buffer to be 5 mM Tris HCl, pH 9, and 0.5 mM EDTA (col. 10, line 17).

Therefore, it would have been prima facie obvious to one of ordinary skill at the time of the invention was made to have modified the method of Boom in view of Shieh or Deggerdal in view of Shieh or Harvey to include the use of the elution buffer described in the method of Su. The ordinary artisan would also have expected that using the elution buffer of Su in the method of Boom or Deggerdal or Harvey with the elution buffer described in Su would have provided equivalent results.

10. Claims 92-93 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boom (5, 804,684) in view of Shieh (US Pat. 6,054,039, April 2000) in view of Rudi et al. (WO 98/51693, November 19, 1998) as applied to Claims above 63-67, 69, 71-73, 82-90, 101-109 or Deggerdal (WO 96/18731) in view of Shieh (US Pat. 6,054,039, April 2000) in view of Rudi et al. (WO 98/51693, November 19, 1998) as applied to Claims above 63-67, 69-80, 82-85, 87-90, 93-96, 101-109 or Harvey et al. (US Pat. 5.939,259, August 1999) in view of Rudi et al. (WO 98/51693, November 19, 1998) as applied to Claims 63-67, 69-73, 82, 84, 87-90, 101-109 above and further in view of Arnold (5,599,667).

Neither Boom, Deggerdal, nor Shieh, nor Harvey specifically teach using polyolefin as a solid support wherein polyolefin is hydrophilic and has a charge.

However, Arnold teaches polycationic solid supports that can be used purification of nucleic acids (see abstract). The polycationic support matrix is taught to include inorganic and organic materials which include glasses, polyolefins and polysaccharides (col.8, lines 55-62).

Therefore, it would have been prima facie obvious to one of ordinary skill at the time of the invention was made to have modified the method of Boom or Deggerdal in view of Shieh or Harvey to include the solid supports of Arnold in order to make the claimed invention as a whole. The ordinary artisan would be motivated to have substituted polyolefins as a solid support in the Boom or Deggerdal or Harvey method because Arnold taught that polyolefins and glass are both suitable for DNA isolation because they meet the same "principle requirement" of "not unduly adsorbing either contaminants or nucleotide probes (col. 8, lines 61-64). Consequently Arnold shows that the silica of Boom or Deggerdal and the polyolefins of the claims are equivalent.

11. Claim 91 is rejected under 35 U.S.C. 103(a) as being unpatentable over Boom (5, 804,684) in view of Shieh (US Pat. 6,054,039, April 2000) in view of Rudi et al. (WO 98/51693, November 19, 1998) or Deggerdal (WO 96/18731) in view of Shieh (US Pat. 6,054,039, April 2000) in view of Rudi et al. (WO 98/51693, November 19, 1998) or Harvey et al. (US Pat. 5.939,259, August 1999) in view of Rudi et al. (WO 98/51693, November 19, 1998) and further in view of Arnold (5,599,6667) as applied to claim 92-93 above, and further in view of Hasebe (5,151,345).

Arnold teaches polycationic solid supports that can be used purification of nucleic acids (see abstract). The polycationic support matrix is taught to include inorganic and organic materials which include glasses, polyolefins and polysaccharides (col.8, lines 55-62).

However, neither Boom or Deggerdal nor Arnold specifically teaches that polyolefin is a mixture of low density polyethylene and polypropylene fibers.

However, Hasebe teaches that "a polyolefin resin is preferred, and low-density polyethylene, high-density polyethylene...or a blend thereof is preferably used"(col. 11, lines 32-39).

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time of the invention was made to combine the methods of Boom or Deggerdal or Harvey and Arnold as discussed above and use the types of polyolefins taught by Hasebe. As Arnold teaches that "polyolefins" may be used in DNA isolation, one of ordinary skill in the art would have been motivated to use a preferred polyolefin resin.

Conclusion

12. **No Claims are allowable over the prior art.**

13. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

A) Harvey et al. Clinical Chem. Vol. 41, pages S108, No. 6, 1995. Harvey teaches impregnating paper with chaotropic salts and their efficiencies. DNA from blood spots collected on guanidine impregnated paper was released in high levels and contained little if any inhibitory substance for PCR. Blood collection paper treated with this chaotrope provides a rapid and reproducible method for the preparation of DNA from died blood spots.

14. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jeanine Goldberg whose telephone number is (571) 272-0743. The examiner can normally be reached Monday-Friday from 7:00 a.m. to 4:00 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (571) 272- 0745.

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The Central Fax Number for official correspondence is (571) 273-8300.

J. Goldberg

Jeanine Goldberg
Primary Examiner
April 18, 2005

Notice of References Cited	Application/Control No. 09/241,636	Applicant(s)/Patent Under Reexamination HEATH ET AL.	
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(54) Title: SOLID–PHASE NUCLEIC ACID ISOLATION

(57) Abstract

The present invention provides a method of isolating nucleic acid from a sample of cells, said method comprising: (a) binding cells in said sample to a solid support to isolate cells from the sample; (b) lysing the isolated cells; and (c) binding nucleic acid released from said lysed cells to said same solid support and a kit for carrying out such a method. The method may advantageously be used to prepare nucleic acid for use in a nucleic acid–based target cell detection method.

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SOLID-PHASE NUCLEIC ACID ISOLATION

The present invention relates to the isolation of nucleic acid, and especially to a method for isolating DNA from cells which combines a solid phase cell isolation step with a solid phase DNA isolation step.

The isolation of nucleic acid is an important step in many biochemical and diagnostic procedures. For example, the separation of nucleic acids from the complex mixtures in which they are often found is frequently necessary before other studies and procedures eg. detection, cloning, sequencing, amplification, hybridisation, cDNA synthesis etc. can be undertaken; the presence of large amounts of cellular or other contaminating material eg. proteins or carbohydrates, in such complex mixtures often impedes many of the reactions and techniques used in molecular biology. In addition, DNA may contaminate RNA preparations and vice versa. Thus, methods for the isolation of nucleic acids from complex mixtures such as cells, tissues etc. are demanded, not only from the preparative point of view, but also in the many methods in use today which rely on the identification of DNA or RNA eg. diagnosis of microbial infections, forensic science, tissue and blood typing, detection of genetic variations etc.

The use of DNA or RNA identification is now widely accepted as a means of distinguishing between different cells or cell types or between variants of the same cell type containing DNA mutations. Thus, HLA typing, which is more commonly carried out by identification of characteristic surface antigens using antibodies, may alternatively be effected by identification of the DNA coding for such antigens. Microbial infection or contamination may be identified by nucleic acid analysis

to detect the target organism, rather than relying on detecting characterising features of the cells of the microorganisms eg. by morphological or biochemical. Genetic variations may be identified by similar means.

In general, DNA or RNA is identified by hybridisation to one or more oligonucleotides under conditions of stringency sufficient to ensure a low level of non specific binding. Commonly, the hybridising nucleotides are used in pairs as primers in the various forms of in vitro amplification now available, primarily the polymerase chain reaction (PCR), but also the Ligase Amplification Reaction (LAR), the Self-Sustained Sequence Replication (3SR) and the Q-beta replicase amplification system. After amplification the DNA may be further characterised by sequencing, eg. by the Sanger method. Amplification and sequencing may be combined.

As mentioned above, all methods generally require an initial nucleic acid isolation step, to separate the nucleic acid from materials eg. protein which may interfere in the hybridisation and amplification techniques which are used.

A range of methods are known for the isolation of nucleic acids, but generally speaking, these rely on a complex series of extraction and washing steps and are time consuming and laborious to perform.

Classical methods for the isolation of nucleic acids from complex starting materials such as blood or blood products or tissues involves lysis of the biological material by a detergent or chaotrope, possibly in the presence of protein degrading enzymes, followed by several extractions with organic solvents eg. phenol and/or chloroform, ethanol precipitation, centrifugations and dialysis of the nucleic acids. Not only are such methods cumbersome and time consuming to perform, but the relatively large number of steps required increases the risk of degradation, sample loss

or cross-contamination of samples where several samples are simultaneously processed.

Improvements in methods for isolating nucleic acids are thus continually being sought, and more recently, other methods have been proposed which rely upon the use of a solid phase. In US-A-5,234,809, for example, is described a method where nucleic acids are bound to a solid phase in the form of silica particles, in the presence of a chaotropic agent such as a guanidinium salt, and thereby separated from the remainder of the sample. WO 91/12079 describes a method whereby nucleic acid is trapped on the surface of a solid phase by precipitation. Generally speaking, alcohols and salts are used as precipitants.

Whilst such methods speed up the nucleic acid separation process, a need still exists for methods which are quick and simple to perform, which enable good yields to be obtained without losses, and in particular which are readily amenable to isolating nucleic acids from cells in mixtures or environments where they may be present at low concentrations, as a preparative first step in isolating nucleic acids from target cells in nucleic-acid based cell detection procedures. The present invention addresses this need. In particular, whilst hybridisation-based techniques such as PCR and other nucleic acid-based methods for detecting microorganisms allow high sensitivity detection of cells in samples, sample preparation ie. the concentration of the target cells and nucleic acid purification, are crucial factors in achieving the high sensitivity and reproducibility of the method. At present, cells are commonly first isolated from the sample by filtration, centrifugation or affinity binding to antibodies attached to a solid phase. After cell concentration in this manner, the DNA is then purified from the concentrated cells, often by classical phenol/chloroform extraction methods as discussed above, with their

attendant disadvantages.

We now propose a novel approach to this problem which integrates cell isolation and nucleic acid purification in a single "step", by using the same solid phase for both cell adsorption and nucleic acid purification. This is achieved by binding the cells to a solid support as a first step. The same solid support is then used under conditions that lyse the bound cells, and then which enable the nucleic acid to bind to the support.

In this manner nucleic acid may be isolated from a sample in a form suitable for amplification or other downstream processes, by a simple and quick to perform procedure which may take less than 45 minutes.

In one aspect, the present invention thus provides a method of isolating nucleic acid from a sample of cells, said method comprising:

(a) binding cells in said sample to a solid support to isolate cells from the sample;

(b) lysing the isolated cells; and

(c) binding nucleic acid released from said lysed cells to said same solid support.

The nucleic acid may be DNA, RNA or any naturally occurring or synthetic modification thereof, and combinations thereof. Preferably however the nucleic acid will be DNA, which may be single or double stranded or in any other form, eg. linear or circular.

The term "cell" is used herein to include all prokaryotic (including archaebacteria) and eukaryotic cells and other viable entities such as viruses and mycoplasmas, and sub-cellular components such as organelles. Representative "cells" thus include all types of mammalian and non-mammalian animal cells, plant cells, protoplasts, bacteria, protozoa and viruses.

The sample may thus be any material containing nucleic acid within such cells, including for example foods and allied products, clinical and environmental

samples. Thus, the sample may be a biological sample, which may contain any viral or cellular material, including all prokaryotic or eukaryotic cells, viruses, bacteriophages, mycoplasmas, protoplasts and organelles. Such biological material may thus comprise all types of mammalian and non-mammalian animal cells, plant cells, algae including blue-green algae, fungi, bacteria, protozoa etc. Representative samples thus include whole blood and blood-derived products such as plasma or buffy coat, urine, faeces, cerebrospinal fluid or any other body fluids, tissues, cell cultures, cell suspensions etc., and also environmental samples such as soil, water, or food samples.

The sample may also include relatively pure or partially purified starting materials, such as semi-pure preparations obtained by other cell separation processes.

Binding of the cells to the solid support may be achieved in any known or convenient manner. For example, non-specific binding of the cells to the support may be achieved by appropriate choice of the solid support and conditions eg. the chemical or physical nature of the surface of the solid support, (eg. hydrophobicity or charge), the pH or composition of the isolation medium etc. The nature of the target cells may also play a role and it has, for example, been shown that certain hydrophobic cells may readily bind non-specifically to hydrophobic surfaces, whereas hydrophilic cells may bind to more hydrophilic surfaces. Negatively charged cells such as B-lymphocytes have also been observed to have a high degree of non-specific binding to weakly-positively charged surfaces. Thus solid supports having appropriately charged surfaces for binding of a desired cell type may be used. Appropriate buffers etc. may be used as media for the cell isolation step to achieve conditions appropriate for cell binding, simply bringing the solid support and the sample into

contact in an appropriate medium. Conveniently, a buffer of appropriate charge, osmolarity etc. may be added to the sample prior to, simultaneously with, or after contact with the solid support.

Advantageously, non-specific binding of cells may be achieved according to the invention by precipitating the cells onto the support using a precipitant, for example by contacting the cells with the support in the presence of alcohol and salt, eg. by adding to the sample, a buffer containing alcohol and salt. The use of alcohol and salt in separation and purification procedures such as precipitation is commonplace and any suitable alcohol or salt used in such procedures, may be used according to the present invention. Thus, conveniently the alcohol may be any alkanol, and lower alkanols such as isopropanol and ethanol have been found to be suitable. Other suitable alcohols include methanol and n-butanol.

The salt may be provided by any convenient source eg. sodium or potassium chloride or acetate, or ammonium acetate. Appropriate concentrations of alcohol and salt may be determined according to the precise system and reagents used. Generally speaking addition of 0.5 to 3 volumes of alcohol eg. 1 volume, to the sample has been found to be suitable. Conveniently the alcohol may be used at concentrations of 50-100% (w/v). The use of salt concentrations of eg. 0.1 to 10.0 M, more particularly 0.1 to 7.0 M, e.g. 0.1 to 3.0 M has been found to be suitable, and conveniently the salt may be included, at the above concentrations in the alcohol solution. Thus, a so-called "cell-binding buffer" may be used containing the alcohol and salt at the desired concentrations. Alternatively, the salt and alcohol may be added separately.

The use of alcohol as precipitant for the cells according to the invention is advantageous for use of the method in clinical diagnostic procedures, since the

use of alcohol to conserve clinical samples is common. Thus, patient samples may simply be added to an alcohol-containing cell-binding buffer, whereby the samples are conserved and ready for purification of the nucleic acid.

As an alternative to precipitation with salt/alcohol, other precipitants may be used, for example polyethylene glycols (PEGs) or other high molecular weight polymers with similar properties, either alone or in combination with salt and/or alcohol. The concentrations of such polymers may vary depending upon the precise system eg. polymer and cell type, but generally concentrations from 1 to 50% (w/v), eg. 2-30% may be used.

Cells with phagocytic activity may be captured by their ability to "bind" or "swallow" a particulate solid phase eg. beads, and thereby can readily be collected. In this case, the cell-containing sample needs simply to be contacted or incubated with the solid phase under appropriate conditions. This kind of cell capture is not dependent on specific binding.

The solid support may also be provided with moieties which assist in the non-specific binding of cells, for example proteins or protein fragments or polypeptides which are bound non-specifically by cells. Thus, for example, a solid support coated with or carrying antibodies will bind cells non-specifically through Fc receptors on the cell surface. Techniques for immobilising antibodies and other proteins or polypeptides on solid surfaces are well known in the art.

Finally, as mentioned above, non-specific cell-binding to solid supports having charged, hydrophobic or hydrophilic surfaces may be achieved by using buffers, often in combination with salt, to achieve pH conditions appropriate for binding. The precise buffers and conditions will vary depending on the type of cell,

solid support etc.

The various components are mixed and simply allowed to stand for a suitable interval of time to allow the cells to bind to the support. The support may then be removed from the solution by any convenient means, which will depend of course on the nature of the support, and includes all forms of withdrawing the support away from the sample supernatant, or vice versa, for example centrifugation, decanting, pipetting etc.

The conditions during this process are not critical, and it has been found convenient, for example, simply to mix the sample with the "cell-binding buffer" in the presence of a solid phase, and allow it to stand at room temperature, eg. for 5 to 30 minutes, eg. 20 minutes before separating. As mentioned above, the reaction time is not critical and as little as 5 minutes may be often enough. However, if convenient, longer periods may be used, eg. 20 minutes to 3 hours, or even overnight. Mixing can be done by any convenient means, including for example simple agitation by stirring or vortexing. Also, if desired, higher or lower temperatures may be used, but are not necessary.

Other optional components in the "cell-binding" composition include high molecular weight polymers eg. PEGs etc., weak uncharged detergents eg. Triton X-100, NP-40 etc, DNAses and other enzymes, as long as they leave the cells intact.

Preferred "cell-binding" compositions may, for example, comprise:

isopropanol, 0.75 M ammonium acetate

75% ethanol, 0.75 M ammonium acetate.

Although non-specific binding of cells is preferred according to the invention, it is also possible to use solid supports which have been modified to permit the selective capture of desired cells containing the nucleic acid. Thus for example, supports carrying antibodies, or other binding proteins eg. lectins,

specific for a desired cell type may be used. This may introduce a degree of selectivity to the isolation of the nucleic acid, since only nucleic acid from a desired target source within a complex mixture may be separated. Thus for example, such a support may be used to separate and remove the desired target cell type etc. from the sample.

The preparation of such selective cell capture matrices is well known in the art and described in the literature.

The solid support may be any of the well known supports or matrices which are currently widely used or proposed for immobilisation, separation etc. These may take the form of particles, sheets, gels, filters, membranes, fibres, capillaries, or microtitre strips, tubes, plates or wells etc.

Conveniently the support may be made of glass, silica, latex or a polymeric material. Preferred are materials presenting a high surface area for binding of the cells, and subsequently, of the nucleic acid. Such supports will generally have an irregular surface and may be for example be porous or particulate eg. particles, fibres, webs, sinters or sieves. Particulate materials eg. beads are generally preferred due to their greater binding capacity, particularly polymeric beads.

Conveniently, a particulate solid support used according to the invention will comprise spherical beads. The size of the beads is not critical, but they may for example be of the order of diameter of at least 1 and preferably at least 2 μm, and have a maximum diameter of preferably not more than 10 and more preferably not more than 6 μm. For example, beads of diameter 2.8 μm and 4.5 μm have been shown to work well.

Monodisperse particles, that is those which are substantially uniform in size (eg. size having a diameter standard deviation of less than 5%) have the advantage that they provide very uniform reproducibility

of reaction. Monodisperse polymer particles produced by the technique described in US-A-4336173 are especially suitable.

Non-magnetic polymer beads suitable for use in the method of the invention are available from Dyno Particles AS (Lillestrøm, Norway) as well as from Qiagen, Pharmacia and Serotec.

However, to aid manipulation and separation, magnetic beads are preferred. The term "magnetic" as used herein means that the support is capable of having a magnetic moment imparted to it when placed in a magnetic field, and thus is displaceable under the action of that field. In other words, a support comprising magnetic particles may readily be removed by magnetic aggregation, which provides a quick, simple and efficient way of separating the particles following the cell and nucleic acid binding steps, and is a far less rigorous method than traditional techniques such as centrifugation which generate shear forces which may disrupt cells or degrade nucleic acids.

Thus, using the method of the invention, the magnetic particles with cells attached may be removed onto a suitable surface by application of a magnetic field eg. using a permanent magnet. It is usually sufficient to apply a magnet to the side of the vessel containing the sample mixture to aggregate the particles to the wall of the vessel and to pour away the remainder of the sample.

Especially preferred are superparamagnetic particles for example those described by Sintef in EP-A-106873, as magnetic aggregation and clumping of the particles during reaction can be avoided, thus ensuring uniform and nucleic acid extraction. The well-known magnetic particles sold by Dynal AS (Oslo, Norway) as DYNABEADS, are particularly suited to use in the present invention.

Functionalised coated particles for use in the

present invention may be prepared by modification of the beads according to US patents 4,336,173, 4,459,378 and 4,654,267. Thus, beads, or other supports, may be prepared having different types of functionalised surface, for example positively or negatively charged, hydrophilic or hydrophobic.

Different cells exhibit different degrees of non-specific binding to different surfaces and supports and it may be advantageous to "titrate" the amount of the solid support (eg. the number of particles) per volume unit, in order to optimise the cell-binding conditions, and determine the optimum support area, eg. particle concentration for a given system.

Following cell binding, the isolated or support-bound cells are lysed to release their nucleic acid. Methods of cell lysis are well known in the art and widely described in the literature and any of the known methods may be used. Different methods may be more appropriate for different cells, but any of the following methods could, for example, be used: detergent lysis using eg. SDS, LiDS or sarkosyl in appropriate buffers; the use of chaotropes such as Guanidium Hydrochloride (GHCl), Guanidium thiocyanate (GTC), sodium iodide (NaI), perchlorate etc; mechanical disruption, such as by a French press, sonication, grinding with glass beads, alumina or in liquid nitrogen; enzymatic lysis, for example using lysozyme, proteinases, pronases or cellulases or any of the other lysis enzymes commercially available; lysis of cells by bacteriophage or virus infection; freeze drying; osmotic shock; microwave treatment; temperature treatment; eg. by heating or boiling, or freezing, eg. in dry ice or liquid nitrogen, and thawing; alkaline lysis. As mentioned above, all such methods are standard lysis techniques and are well known in the art, and any such method or combination of methods may be used.

Conveniently, lysis may be achieved according to

the present invention by using chaotropes and/or detergents. For example, in the case of bacterial cells, the combination of a chaotrope with a detergent has been found to be particularly effective. An exemplary suitable lysis agent thus includes a chaotrope such as GTC or GHCl and a detergent such as SDS or Sarkosyl. The lysis agents may be supplied in simple aqueous solution, or they may be included in a buffer solution, to form a so-called "lysis buffer". Any sutable buffer may be used, including for example Tris, Bicine, Tricine and phosphate buffers. Alternatively the lysis agents may be added separately. Suitable concentrations and amounts of lysis agents will vary according to the precise system, nature of the calls etc. and may be appropriately determined, but concentrations of eg. 2M to 7M chaotropes such as GTC GHCl, NaI or perchlorate may be used, 0.1M to 1M alkaline agents such as NaOH, and 0.1 to 50% (w/v) eg. 0.5 to 15% detergent. Thus, an example of a suitable representative lysis buffer includes an aqueous solution of 4M GTC, 1% (w/v) sarkosyl.

To carry out the method of the invention, the isolated, support-bound cells, may conveniently be removed or separated from the remainder of the sample, thereby concentrating or enriching the cells. Thus the cell binding step serves to enrich the cells or to concentrate them in a smaller volume than the initial sample. Lysis then may conveniently be achieved by adding an appropriate lysis buffer containing the desired lysis agents or by subjecting the isolated cells to the desired lysis conditions. For example, in the case of simply adding a lysis buffer containing appropriate lysis agents, the isolated cells may simply be incubated in the presence of the lysis buffer for a suitable interval to allow lysis to take place. Different incubation conditions may be appropriate for different lysis systems, and are known in the art. For

example for a detergent and/or chaotrope containing lysis buffer, incubation may take place at room temperature or at higher temperatures eg. 37°C or 65°C. Likewise, time of incubation may be varied from a few minutes eg. 5 or 10 minutes to hours, eg. 1 to 2 hours. In the case of GTC/sarkosyl lysis buffers and bacterial cells, incubation at eg. 65°C for 10-20 minutes has been found to be appropriate, but this may of course be varied according to need. For enzymatic lysis, eg. using proteinase K etc, longer treatment times may be required, eg. overnight.

Following lysis, the released nucleic acid is bound to the same support to which the lysed cells are bound. This nucleic acid binding may be achieved in any way known in the art for binding nucleic acid to a solid support. Conveniently, the nucleic acid is bound non-specifically to the support ie. independently of sequence. Thus, for example the released nucleic acid may be precipitated onto the support using any of the known precipitants for nucleic acid, eg. alcohols, alcohol/salt combinations, polyethylene glycols (PEGs) etc. Precipitation of nucleic acids onto beads in this manner is described for example in WO 91/12079. Thus, salt may be added to the support and released nucleic acid in solution, followed by addition of alcohol which will cause the nucleic acid to precipitate. Alternatively, the salt and alcohol may be added together, or the salt may be omitted. As described above in relation to the cell binding step, any suitable alcohol or salt may be used, and appropriate amounts or concentrations may readily be determined.

Alternative non-specific nucleic acid-binding techniques include the use of detergents as described in WO 96/18731 of Dynal AS (the so-called "DNA Direct" procedure), and the use of chaotropes and a nucleic acid-binding solid phase such as silica particles as described by Akzo N.V. in EP-A-0389063.

Ionic binding of the nucleic acid to the support may be achieved by using a solid support having a charged surface, for example a support coated with polyamines.

The support which is used in the method of the invention may also carry functional groups which assist in the specific or non-specific binding of nucleic acids, for example DNA binding proteins eg. leucine zippers or histones or intercalating dyes (eg. ethidium bromide or Hoechst 42945) which may be coated onto the support.

Likewise, the support may be provided with binding partners to assist in the selective capture of nucleic acids. For example, complementary DNA or RNA sequences, or DNA binding proteins may be used, or viral proteins binding to viral nucleic acid. The attachment of such proteins to the solid support may be achieved using techniques well known in the art.

A convenient method of precipitating the nucleic acid according to the invention is by adding a precipitant, eg. alcohol, to the mixture containing the support and lysed cells. Thus, an appropriate volume of alcohol, eg. 100% or 96% ethanol, may simply be added to the mixture, and incubated for a time period sufficient to allow the released nucleic acid to become bound to the support. The incubation conditions for this step are not critical and may simply comprise incubating at 5-10 minutes at room temperature. However, the length of time may be varied, and temperature increased according to choice.

Although not necessary, it may be convenient to introduce one or more washing steps to the isolation method of the invention, for example following the nucleic acid binding step. Any conventional washing buffers or other media may be used. Generally speaking, low to moderate ionic strength buffers are preferred eg. 10 mM Tris-HCl at pH 8.0/10mM NaCl. Other standard

washing media, eg. containing alcohols, may also be used, if desired, for example washing with 70% ethanol.

The use of magnetic particles permits easy washing steps simply by aggregating the particles, removing the nucleic acid binding medium, adding the washing medium and reaggregating the particles as many times as required.

Following the nucleic acid isolation process and any optional washing steps which may be desired, the support carrying the bound nucleic acid may be transferred eg. resuspended or immersed into any suitable medium eg. water or low ionic strength buffer. Depending on the support and the nature of any subsequent processing desired, it may or may not be desirable to release the nucleic acid from the support.

In the case of a particulate solid support such as magnetic or non-magnetic beads, this may in many cases be used directly, for example in PCR or other amplifications, without eluting the nucleic acid from the support. Also, for many DNA detection or identification methods elution is not necessary since although the DNA may be randomly in contact with the bead surface and bound at a number of points by hydrogen bonding or ionic or other forces, there will generally be sufficient lengths of DNA available for hybridisation to oligonucleotides and for amplification.

However, if desired, elution of the nucleic acid may readily be achieved using known means, for example by heating, eg. to 65°C for 5 to 10 minutes, following which the support may be removed from the medium leaving the nucleic acid in solution. Such heating is automatically obtained in PCR by the DNA denaturation step preceding the cycling program.

If it is desired to remove RNA from DNA, this may be achieved by destroying the RNA before the DNA separation step, for example by addition of an RNAase or an alkali such as NaOH.

An advantage of the present invention, is that it is quick and simple to perform, and with an appropriate combination of cell-binding, lysis and nucleic acid binding steps, provides a method which reliably and simply yields isolated nucleic acid in a short period of time, in many cases, less than one hour, or even less than 45 minutes. The simplicity of the method allows for high throughput of samples. Concomitantly, the cell-binding step, results in an enrichment or concentration of the cells, thereby improving the nucleic acid isolation process.

The invention is advantageously amenable to automation, particularly if particles, and especially, magnetic particles are used as the support.

As mentioned above, the method of the invention has particular utility as a preliminary first step to prepare nucleic acid for use in nucleic acid-based detection procedures.

Thus, a further aspect of the present invention is the use of the nucleic acid isolation method as hereinbefore defined in the preparation of nucleic acid for use in a nucleic acid-based target cell detection method.

Alternatively viewed, this aspect of the invention provides a method for detecting the presence or absence of a target cell in a sample, said method comprising:

(a) binding cells in said sample to a solid support to isolate cells from the sample;

(b) lysing the isolated cells;

(c) binding nucleic acid released from said lysed cells to said same solid support; and

(d) detecting the presence or absence of nucleic acid characteristic of said target cells within said bound nucleic acid.

As mentioned above, advantageously the bound nucleic acid need not be eluted or removed from the support prior to carrying out the detection step,

although this may be performed if desired. Whether or not the nucleic acid is eluted may also depend on the particular method which was used in the nucleic acid binding step. Thus certain nucleic acid-binding procedures will bind the nucleic acid more tightly than others. In the case of DNA-binding using detergents (eg. by DNA Direct) for example, the nucleic acid will elute from the solid support when an elution buffer or other appropriate medium is introduced. Nucleic acid bound by means of a precipitant such as alcohol or a chaotrope will remain more tightly bound and may not elute when placed in a buffer medium, and may require heating to be eluted.

Thus, the support-bound nucleic acid may be used directly in a nucleic acid based detection procedure, especially if the support is particulate, simply by resuspending the support in, or adding to the support, a medium appropriate for the detection step. Either the nucleic acid may elute into the medium, or as mentioned above, it is not necessary for it to elute.

A number of different techniques for detecting nucleic acids are known and described in the literature and any of these may be used according to the present invention. At its simplest the nucleic acid may be detected by hybridisation to a probe and very many such hybridisation protocols have been described (see eg. Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press, Cold Spring Harbor, NY). Most commonly, the detection will involve an *in situ* hybridisation step, and/or an in *vitro* amplification step using any of the methods described in the literature for this. Thus, as mentioned, techniques such as LAR, 3SR and the Q-beta-replicase system may be used. However, PCR and its various modifications eg. the use of nested primers, will generally be the method of choice (see eg. Abramson and Myers, 1993, Current Opinion in Biotechnology, 4: 41-47 for a review of

nucleic acid amplification technologies).

Other detection methods may be based on a sequencing approach, for example, the minisequencing approach as described by Syvänen and Söderlund, 1990, Genomics, 8: 684-692.

In amplification techniques such as PCR, the heating required in the first step to melt the DNA duplex may release the bound DNA from the support. Thus, in the case of a subsequent detection step, such as PCR, the support bound nucleic acid may be added directly to the reaction mix, and the nucleic acid will elute in the first step of the detection process. The entire isolated support bound nucleic acid sample obtained according to the invention may be used in the detection step, or an aliquot.

The results of the PCR or other detection step may be detected or visualised by many means, which are described in the art. For example the PCR or other amplification products may be run on an electrophoresis gel eg. an ethidium bromide stained agarose gel using known techniques. Alternatively, the DIANA system may be used, which is a modification of the nested primer technique. In the DIANA (Detection of Immobilised Amplified Nucleic Acids) system (see Wahlberg et al., Mol. Cell Probes 4: 285(1990)), the inner, second pair of primers carry, respectively, means for immobilisation to permit capture of amplified DNA, and a label or means for attachment of a label to permit recognition. This provides the dual advantages of a reduced background signal, and a rapid and easy means for detection of the amplified DNA.

The amplified nucleic acid may also be detected, or the result confirmed, by sequencing, using any of the many different sequencing technologies which are now available, eg. standard sequencing, solid phase sequencing, cyclic sequencing, automatic sequencing and minisequencing.

Advantageously, it has been found that isolated cells may be kept in a "cell-binding" buffer according to the invention eg. a salt/alcohol buffer for at least one week at room temperature with no detectable loss of sensitivity in a subsequent nucleic acid detection step. Such stability is an advantage in field situations.

The various reactants and components required to perform the methods of the invention may conveniently be supplied in kit form. Such kits represent a further aspect of the invention.

At its simplest, this aspect of the invention provides a kit for isolating nucleic acid from a sample comprising:

(a) a solid support;

(b) optionally, means for binding cells to said solid support;

(c) means for lysing said cells; and

(d) means for binding nucleic acid released from said lysed cells to said same solid support.

The various means (b), (c) and (d) may be as described and discussed above, in relation to the method of the invention.

A further optional component is (e), means for detecting the presence or absence of nucleic acid characteristic of a target cell within said band nucleic acid. As discussed above, such means may include appropriate probe or primer oligonucleotide sequences for use in hybridisation and/or amplification-based detection techniques.

Optionally further included in such a kit may be buffers, salts, polymers, enzymes etc.

The invention will now be described in more detail in the following non-limiting Examples with reference to the drawings in which:

Figure 1 shows the results on EtBr-stained agarose gel electrophoresis of the separation of PCR amplification products of DNA obtained according to the

invention from cells of 5 cyanobacterial species. Lanes 1 to 7 correspond to samples 1 to 7 as described in Example 1.

Example 1

Materials

Sample 1; *Planktothrix rubescens* NIVA-CYA 1, sample 2; *Planktothrix agardhii* NIVA-CYA 29, sample 3; *Planktothrix rubescens* NIVA-CYA 55, sample 4; *Planktothrix mougeotii* NIVA-CYA 56/1, sample 5; *Planktothrix agardhii* NIVA-CYA 116, sample 6; negative control on cell and DNA purification reagents and sample 7; negative control on PCR reagents.

Cell and DNA isolation protocol:

0.5 ml of water containing approximately 10^5 cells as mixed with 20 µl beads (1 µg/ml) and 0.5 ml cell binding buffer (isopropanol, 0.75 M NH_4Ac) in a microcentrifuge tube. The mixture was incubated at room temperature for 20 minutes, then the tube was placed in a MPC-E magnet (Dynal A.S.) for 2 minutes. The supernatant was carefully removed. 50 µl 4 M GTC, 1% sarkosyl was added and incubated at 65°C for 10 minutes. Then 200 µl of 96% EtOH was added and the incubation continued for 5 minutes at room temperature. The beads were attracted to the tube wall with the magnet and the supernatant removed. The complex was washed twice with 500 µl 70% EtOH. All of the ethanol was removed and 50 µl water added. To remove residual ethanol the tubes were incubated at 65°C for 10 minutes with an open lid.

PCR amplification:

The region between the genes coding for RuBisCo large (Rbcl) and small subunit (Rbcs) was amplified. Amplifications were done using the GeneAmp 2400 PCR

system (Perkin Elmer) in 50 µl volumes containing 10 pmol primers (CW) 5'CGTAGCTTCCGGTGGTATCCACGT3' and (DF) 5'GGGCARYTTCCACAKNGTCCA3', 200 µM dNTP, 10 mM Tris-HCl (pH8.8) 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 1U DynaZyme thermostable DNA polymerase (Finnzymes Oy) and 5 µl of the bead/DNA solution. The PCR program used has an initial denaturation step at 94°C for 4 minutes, then cycling with the parameters; 94°C for 30 seconds, 40°C for 30 seconds and 72°C for 2 minutes for 2 cycles, then 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 2 minutes for 40 cycles. Finally, an extension step for 7 minutes was included. By DNA sequencing the amplified fragments were verified to be the region between RuBisCo large (Rbcl) and small (Rbcs) subunit.

Gel:

10 µl of the amplified products from each of samples 1 to 7 were run on a 1.5% EtBr stained agarose gel for 30 minutes at 100 volts. The molecular weight standard was ϕX 174 HaeIII digested DNA. The results are shown in Figure 1.

Results:

Figure 1 clearly shows that the cells from all the samples 1 to 5 could be detected. Based on the PCR results obtained, as visualised on the EtBr stained agarose gel, the detection limit was estimated to be 10-100 cells/ml.

Claims

1. A method of isolating nucleic acid from a sample of cells, said method comprising:

(a) binding cells in said sample to a solid support to isolate cells from the sample;

(b) lysing the isolated cells; and

(c) binding nucleic acid released from said lysed cells to said same solid support.

2. The method of claim 1, wherein said nucleic acid is DNA.

3. The method of claim 1 or claim 2, wherein in step (a) the cells are precipitated onto the support using a precipitant.

4. The method of claim 3, wherein the precipitant comprises alcohol and salt.

5. The method of claim 1 or claim 2, wherein in step (a) the cells bind to the support by virtue of cell-binding moieties provided on or in the support.

6. The method of claim 5 wherein said cell-binding moieties permit selective binding of target cells.

7. The method of any one of claims 1 to 6 wherein the solid support is particulate.

8. The method of claim 7, wherein the support comprises magnetic beads.

9. The method of any one of claims 1 to 8, wherein in step (b), the cells are lysed using a detergent, and/or a chaotrope.

10. The method of any one of claims 1 to 9, wherein in step (c) the nucleic acid is bound non-specifically to the support.

11. The method of claim 10, wherein the nucleic acid is precipitated onto the support using a precipitant.

12. The method of claim 11, wherein the precipitant comprises alcohol, and optionally salt, and/or a detergent.

13. The method of any one of claims 1 to 9, wherein in step (c) the nucleic acid binds to the support by virtue of binding partners provided on the support to assist in the selective capture of nucleic acids.

14. The method of any one of claims 1 to 13, wherein the bound nucleic acid is eluted from the support.

15. The use of the nucleic acid isolation method as defined in any one of claims 1 to 14, in the preparation of nucleic acid for use in a nucleic acid-based target cell detection method.

16. A method for detecting the presence or absence of a target cell in a sample, said method comprising:

(a) binding cells in said sample to a solid support to isolate cells from the sample;

(b) lysing the isolated cells;

(c) binding nucleic acid released from said lysed cells to said same solid support; and

(d) detecting the presence or absence of nucleic acid characteristic of said target cells within said bound nucleic acid.

17. The method of claim 16, wherein said detection step (d) comprises *in situ* hybridisation, and/or *in vitro*

amplification and/or nucleic acid sequencing.

18. A kit for isolating nucleic acid from a sample comprising:

(a) a solid support;

(b) optionally, means for binding cells to said solid support;

(c) means for lysing said cells; and

(d) means for binding nucleic acid released from said lysed cells to said same solid support;

wherein said solid support (a) and means (b) to (d) are as defined in any one of claims 1 to 13.

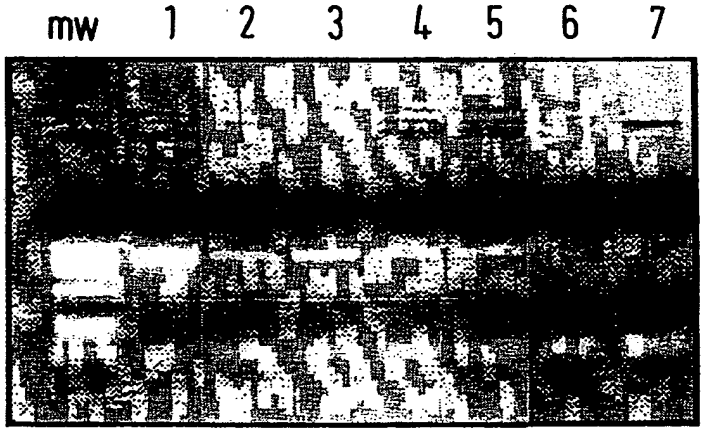

FIG. 1

INTERNATIONAL SEARCH REPORT

rnational Application No
PCT/GB 98/01358

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07H1/08

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 07863 A (DIAGEN INST MOLEKULARBIO) 14 May 1992 see the whole document	1,2,5-7, 9-12,14
A	EP 0 605 003 A (VICAM LP) 6 July 1994 see column 7, line 25 - column 9, line 7 see column 10, line 10 - column 11, line 38 see examples 1-3 see claims 1-8,17	1,2,16, 17
A	WO 97 09600 A (MEDICAL RES COUNCIL ;NEILL IAN KENNETH O (GB); LOKTIONOV ALEXANDRE) 13 March 1997 see page 4, line 23 - line 32 see page 13, line 21 - page 14, line 7 see page 28, line 1 - page 30, line 38	1,2,16, 18

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/01358

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	M.J.DAVIES ET AL.: "Magnetic Solid Phase Supports for Affinity Purification of Nucleic Acids." SPECIAL PUBLICATIONS OF THE ROYAL SOCIETY OF CHEMISTRY. 158 SEPARATIONS FOR BIOTECHNOLOGY, no. 158, 1994, pages 152-158, XP002081359 see abstract	1,2,7,8

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